WEST Search History

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DATE: Thursday, February 15, 2007

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=YES; OP=ADJ		
☐	L8	gene variants same lipase	8
☐	L7	gene variants lipase	3
☐	L6	asn166tyr	0
☐	L5	N166Y	0
☐	L4	lipase and N166Y	0
☐	L3	bacillus and N166Y	0
☐	L2	bacillus same lipase variant?	20
☐	L1	bacillus near 5 lipase variant?	0

END OF SEARCH HISTORY

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=> file medline hcaplus biosis biotechds scisearch embase
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

FILE 'MEDLINE' ENTERED AT 15:00:41 ON 15 FEB 2007

FILE 'HCAPLUS' ENTERED AT 15:00:41 ON 15 FEB 2007
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FILE 'SCISEARCH' ENTERED AT 15:00:41 ON 15 FEB 2007
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FILE 'EMBASE' ENTERED AT 15:00:41 ON 15 FEB 2007
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=> s lipase gene variant?
L1          69 LIPASE GENE VARIANT?

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2          31 DUP REM L1 (38 DUPLICATES REMOVED)

=> s l2 and bacillus
L3           1 L2 AND BACILLUS

=> d l3 ibib ab

L3    ANSWER 1 OF 1  BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-19056  BIOTECHDS
TITLE:            Novel lipase gene variants that
                  is thermostable, organic solvent resistant and high pH
                  tolerant, useful in household detergents and laundry industry
      ;
                     plasmid-mediated gene transfer and expression in
                     Escherichia coli for recombinant enzyme production and
                     purification
AUTHOR:           RAO N M; ACHARYA P
PATENT ASSIGNEE:  COUNCIL SCI and IND RES
PATENT INFO:      WO 2004067705 12 Aug 2004
APPLICATION INFO: WO 2004-IN22 29 Jan 2004
PRIORITY INFO:    IN 2003-75 30 Jan 2003; IN 2003-75 30 Jan 2003
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2004-594198 [57]
AB    DERWENT ABSTRACT:
      NOVELTY - A thermostable, organic solvent resistant and high pH tolerant
      lipase gene variants (I) comprising five
      sequences (S1-S5) of 552 nucleotides fully defined in the specification,
      with a molecular weight of 19443, 19515, 19456.9, 19487 and 19470.9,
      respectively, is new.
           DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the
      following: (1) an expression system (II) for (I), comprising (I) present
      in the vector pJO290; and (2) preparing (II), involves isolating and
      purifying lipase gene from Bacillus subtilis, cloning isolated
      lipase gene in vector pJO290, generating gene variants from isolated
      lipase gene by random mutagenesis and site-directed mutagenesis using
```

forward primer JOF having a sequence of 5'-CGCCAGGGTTTTCCCAGTCACGAC-3', and reverse primer JOR having a sequence of 5'-TGACACAGGAAACAGCTATGAC-3', cloning the gene variants obtained in generating step in plasmid vector pJO290, and ligating the cloned gene variants in Escherichia coli JM109.

BIOTECHNOLOGY - Preferred Gene Variant: In (I), the gene variants are thermostable in the temperature range of 45-95 degreesC, preferably 55-90 degreesC. The T(1/2) value of (I) is in the range of 6-685, preferably 7-677. The Km value is in the range of 0.50-2.5 mM, preferably 0.63-1.96 mM. The K(cat) value is in the range of 4.5x10 to the power -2 to 8.5x10 to the power -2/minute, preferably 5x10 to the power -2 to 8.1x10 to the power -2/minute. The K(cat)/Km value is 4x10 to the power -2 to 10x10 to the power -2/minute, preferably 4.1x10 to the power -2 to 9.7x10 to the power -2/minute. The gene variants are resistant to organic solvents such as acetonitrile, isopropanol, dimethyl sulfoxide and dimethyl formide, preferably acetonitrile. The residual activity of (I) is 25-100%, preferably 28.7-85.5% in presence of acetonitrile. The gene variants have inherent ability to withstand high pH in the range of 9 to 13, ability to withstand damaging surfactants and enzymes comprising groups of linear alkyl benzene sulfonates, proteases and their compounds.

USE - (I) is useful in household detergents and laundry industry.

ADVANTAGE - (I) has improved thermostability at a temperature of 45-95 degreesC, preferably 55-90 degreesC. (I) are resistant to organic solvents such as acetonitrile, isopropanol, dimethyl sulfoxide, or dimethyl formide, preferably acetonitrile. (I) has inherent ability to withstand high pH in the range of 9 to 13, ability to withstand damaging surfactants and enzymes comprising groups of linear alkyl benzene sulfonates, proteases and their compounds (claimed). The thermostability of (I) has increased 200 folds.

EXAMPLE - The lipase gene (isolated from Bacillus subtilis) was mutagenized by error-prone PCR (Cadwell and Joyce, 1992). Primers JOF (5'-CGCCAGGGTTTTCCCAGTCACGAC-3') and JOR (5'-TGACACAGGAAACAGCTATGAC-3') flank the gene beyond the EcoRI and BamHI sites present on the plasmid. Error-prone PCR was carried out in a 100 microl reaction volume containing 20 femtomoles of the plasmid pJO290-lip, 50 pmoles each of primers JOF and JOR, 100 mM Tris.Cl (pH 8.3 at 25 degreesC), 500 mM KCl, 0.1% gelatin (w/v), 7 mM MgCl2, 0.25 mM MnCl2, 1 mM each of dTTP and dCTP, 0.2 mM each of dATP and dCTP and 5 units Taq DNA polymerase. After an initial denaturation of 3 minutes at 94degreesC, the following steps were repeated for 30 cycles in a thermal cycler: 1 minute at 94 degreesC, 1 minute at 45 degreesC and 1 minute at 72 degreesC. The amplified product was precipitated with ethanol, eluted from a 1% agarose gel and digested with EcoRI and BamHI. The digested product was again eluted from a 1% agarose gel and ligated with pJO290 digested with EcoRI and BamHI. The ligation mix was transformed into Escherichia coli JM109 and selection was done on LB-agar supplemented with 25 microg/ml chloramphenicol and 0.2% glucose. Site-directed mutagenesis was carried out on the lipase gene cloned in pET-21b by a modified PCR technique (Chen and Arnold, 1991). For each substitution an oligonucleotide containing the desired mutation was used as the primer (mismatch primer) to initiate chain extension between the 5' and 3' PCR primers. In the first PCR, the mismatch primer and the 3' primer were used to generate a DNA fragment containing the new base substitution. The fragment was separated from the template and primers by agarose gel electrophoresis, purified and used as a new 3' primer in a second PCR with the 5' primer to generate full length product, which was cloned into pET-21b for expression of the mutant protein. (51 pages)

```
=> s bacillus lipase and (mutant? or variant? or mutation?)
L4          18 BACILLUS LIPASE AND (MUTANT? OR VARIANT? OR MUTATION?)

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5          10 DUP REM L4 (8 DUPLICATES REMOVED)
```

```
=> s l5 and N166Y
L6            0 L5 AND N166Y

=> s l5 and A69V
L7            0 L5 AND A69V

=> d l5 1-10 ibib ab

L5   ANSWER 1 OF 10  HCAPLUS  COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER:        2006:930421  HCAPLUS
DOCUMENT NUMBER:         146:1486
TITLE:                   Heat-inducible autolytic vector for high-throughput
                         screening
AUTHOR(S):               Xu, Lihua; Li, Shuang; Ren, Chuan; Cai, Zhen; Lin,
                         Zhanglin
CORPORATE SOURCE:        Tsinghua University, Beijing, Peop. Rep. China
SOURCE:                  BioTechniques (2006), 41(3), 319-323
                         CODEN: BTNQDO; ISSN: 0736-6205
PUBLISHER:               Informa Healthcare USA, Inc.
DOCUMENT TYPE:           Journal
LANGUAGE:                English
AB   In directed evolution, a high-throughput screening system is often a
     prerequisite for sampling the enzyme variants. When the target
     enzyme is expressed intracellularly, for example when Escherichia coli is
     used as the host, chem. or enzymic disruption of cell membrane is often
     required in many cases, which can be tedious, time-consuming, and costly.
     In this study, a set of heat-inducible autolytic vectors were constructed
     to solve this problem, in which the SRRz lysis gene cassette from
     bacteriophage .lambda. was placed downstream of heat-inducible promoters,
     .lambda. cI857/pR promoter and its mutant, cI857/pR(M). The
     artificial autolytic units were inserted into the backbone of pUCI8 (away
     from the multiple cloning sites). For the wild promoter, cI857/pR, the
     SRRz lysis cassette was expressed by temp. up-shift from 28.degree. to
     38.degree.C, and the lysis efficiency of transformed bacterial cells was
     found to be consistent and could reach 96.3% as measured by the reporter
     .beta.-galactosidase assay. In order to obtain a higher cell growth rate,
     the mutant promoter cI857/pR(M) was utilized to allow bacteria
     growth at 35.degree.C and lysis at 42.degree.C. However, this
     heat-inducible system showed significant inconsistency in terms of lysis
     efficiency. Bacillus subtilis 168 lipase A gene was further inserted into
     the multiple cloning sites of the autolytic vector contg. cI857/pR, and
     93.7% of the expressed lipase activity was found in the culture medium
     upon heat induction, demonstrating the utility of the vector for
     expression and rapid extracellular assay of heterologous enzymes.
REFERENCE COUNT:         17    THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS
                               RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5   ANSWER 2 OF 10     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:    2006053532     MEDLINE
DOCUMENT NUMBER:     PubMed ID: 16342303
TITLE:               Directed evolution of Bacillus subtilis lipase A by use of
                     enantiomeric phosphonate inhibitors: crystal structures and
                     phage display selection.
AUTHOR:              Droge Melloney J; Boersma Ykelien L; van Pouderoyen Gertie;
                     Vrenken Titia E; Ruggeberg Carsten J; Reetz Manfred T;
                     Dijkstra Bauke W; Quax Wim J
CORPORATE SOURCE:    Dept. of Pharmaceutical Biology, University of Groningen,
                     A. Deusinglaan 1, 9713 AV Groningen, The Netherlands.
SOURCE:              Chembiochem : a European journal of chemical biology, (2006
                     Jan) Vol. 7, No. 1, pp. 149-57.
                     Journal code: 100937360. ISSN: 1439-4227.
PUB. COUNTRY:        Germany: Germany, Federal Republic of
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
```

ENTRY MONTH: 200604
ENTRY DATE: Entered STN: 28 Jan 2006
Last Updated on STN: 7 Apr 2006
Entered Medline: 6 Apr 2006

AB Phage display can be used as a protein-engineering tool for the selection of proteins with desirable binding properties from a library of mutants. Here we describe the application of this method for the directed evolution of Bacillus subtilis lipase A, an enzyme that has important properties for the preparation of the pharmaceutically relevant chiral compound 1,2-O-isopropylidene-sn-glycerol (IPG). PCR mutagenesis with spiked oligonucleotides was employed for saturation mutagenesis of a stretch of amino acids near the active site. After expression of these mutants on bacteriophages, dual selection with (S)-(+)- and (R)-(-)-IPG stereoisomers covalently coupled to enantiomeric phosphonate suicide inhibitors (SIRAN Sc and Rc inhibitors, respectively) was used for the isolation of variants with inverted enantioselectivity. The mutants were further characterised by determination of their Michaelis-Menten parameters. The 3D structures of the Sc and Rc inhibitor-lipase complexes were determined and provided structural insight into the mechanism of enantioselectivity of the enzyme. In conclusion, we have used phage display as a fast and reproducible method for the selection of Bacillus lipase A mutant enzymes with inverted enantioselectivity.

L5 ANSWER 3 OF 10 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2005:395431 HCAPLUS
DOCUMENT NUMBER: 142:425882
TITLE: Glyceride substrates for screening of 2-specific lipases and identification and cloning of the lipase from Pseudozyma species
INVENTOR(S): Platt, Dorit; Shulman, Avidor; Farkash, Orly; Kaiyal, Muhammad; Katz, Ariel; Basheer, Sobhi
PATENT ASSIGNEE(S): Enzymotec Ltd., Israel
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005040334	A2	20050506	WO 2004-IL966	20041024
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: IL 2003-158576 A 20031023
US 2004-553555P P 20040317

AB The present invention relates to a novel lipase having enhanced selectivity towards the sn-2 position of glycerides and to one-step catalytic reactions using the lipase. The one-step catalytic reaction are directed to the synthesis and/or hydrolysis of glycerides and derivs. thereof at the sn-2 position. The present invention further relates to methods of screening for lipases with significantly enhanced sn-2 regioselectivity, using substrates such as 2-palmitoyl-1,3-dihexadecyloxypropane, which have non-hydrolyzable alkyl moieties bound by ether bonds at the 1 and 3 positions. The lipase cloned from Pseudozyma

species has selectivity towards the sn-2 position of glycerides that is superior to any com. lipase, esp. pronounced in reactions carried out in org. media such as alcoholysis or interesterification reactions. Novozym 868 is also discovered to have some activity toward the sn-2 position with 1,2-dipalmitin. Mutagenesis of lipase T-6 from Bacillus stearothermophilus provides several mutants with superior selectivity (.apprx.57% 1,2 and 43% 1,3) compared to wild-type lipases.

L5 ANSWER 4 OF 10 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2005:1217401 HCAPLUS
DOCUMENT NUMBER: 144:483450
TITLE: Stabilization of Bacillus subtilis Lipase A by increasing the residual packing
AUTHOR(S): Abraham, Tajo; Pack, Seung Pil; Yoo, Young Je
CORPORATE SOURCE: School of Chemical and Biological Engineering, Seoul National University, Seoul, 151-742, S. Korea
SOURCE: Biocatalysis and Biotransformation (2005), 23(3/4), 217-224
CODEN: BOBOEQ; ISSN: 1024-2422
PUBLISHER: Taylor & Francis Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Introduction of well-packed residues to the interior of a protein structure could be considered as a stabilization strategy since the redn. of buried cavities might stabilize protein structure. In this study, the less-packed residues with no water-contact were selected as target sites for increasing residual packing. When Lipase A from Bacillus subtilis (179 amino acids) was used as a model system, 43 less-packed residues were initially considered by analyzing their residual packing value and residual exposure ratio. Among the 43 residues, small amino acids such as Gly and Ala were chosen as target sites. Packing increases of Ala to Val and Gly to Ala were estd., by mol. modeling, to give 0.5368.apprx.0.7433 kcal mol-1 stabilization. Mutants of Lipase A such as A38V, A75V, G80A, A105V A146V, and G172A were obtained via protein engineering. Thermostability assays revealed that A38V, G80A and G172V were the most stable mutants. This procedure for selecting the target residues for improved thermostability of Lipase A could be applied for improving the thermostability of other proteins and enzymes.
REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 5 OF 10 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2003:503485 BIOSIS
DOCUMENT NUMBER: PREV200300505105
TITLE: Extracellular lipases from Bacillus subtilis: Regulation of gene expression and enzyme activity by amino acid supply and external pH.
AUTHOR(S): Eggert, Thorsten; Brockmeier, Ulf; Droge, Melloney J.; Quax, Wim J.; Jaeger, Karl-Erich [Reprint Author]
CORPORATE SOURCE: Institut fuer Molekulare Enzymtechnologie, Heinrich-Heine Universitaet Duesseldorf, Forschungszentrum Juelich, D-52426, Juelich, Germany
karl-erich.jaeger@fz-juelich.de
SOURCE: FEMS Microbiology Letters, (29 August 2003) Vol. 225, No. 2, pp. 319-324. print.
CODEN: FMLED7. ISSN: 0378-1097.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 29 Oct 2003
Last Updated on STN: 29 Oct 2003
AB Bacillus subtilis secretes two lipases LipA and LipB into the culture medium. Both enzyme genes were differentially expressed depending on the growth conditions as determined by activity assays and Western blotting in B. subtilis mutant strains lipA, lipB, and the corresponding lipA/lipB double mutant. In minimal medium, LipA was produced

at wild-type level in a lipB mutant, however, no LipB protein was detected in a lipA mutant. Interestingly, LipA was produced and secreted at wild-type level in rich medium, but the enzyme remained enzymatically inactive, presumably being caused by a shift of the growth medium to acid pH. Furthermore, expression of the lipase genes was studied using transcriptional fusions with the lacZ reporter gene. The expression of lipA was repressed by high amino acid concentrations, whereas the lipB gene expression remained unaffected.

L5 ANSWER 6 OF 10 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2003017926 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12523966
TITLE: Binding of phage displayed Bacillus subtilis lipase A to a phosphonate suicide inhibitor.
AUTHOR: Droge Melloney J; Ruggeberg Carsten J; van der Sloot Almer M; Schimmel Judith; Dijkstra Dolf Swaving; Verhaert Raymond M D; Reetz Manfred T; Quax Wim J
CORPORATE SOURCE: Department of Pharmaceutical Biology, University Centre for Pharmacy, University of Groningen, Antonius Deusinglaan 1, NL-9713 AV Groningen, The Netherlands.
SOURCE: Journal of biotechnology, (2003 Feb 27) Vol. 101, No. 1, pp. 19-28.
Journal code: 8411927. ISSN: 0168-1656.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200309
ENTRY DATE: Entered STN: 14 Jan 2003
Last Updated on STN: 25 Sep 2003
Entered Medline: 24 Sep 2003

AB Phage display can be used as a protein engineering tool to select proteins with desirable binding properties from a library of randomly constructed mutants. Here, we describe the development of this method for the directed evolution of Bacillus subtilis lipase A, an enzyme that has marked properties for the preparation of pharmaceutically relevant chiral compounds. The lipase gene was cloned upstream of the phage g3p encoding sequence and downstream of a modified g3p signal sequence. Consequently, the enzyme was displayed at the surface of bacteriophage fd as a fusion to its minor coat protein g3p. The phage-bound lipase was correctly folded and fully enzymatically active as determined from the hydrolysis of p-nitrophenylcaprylate with K(m)-values of 0.38 and 0.33 mM for the phage displayed and soluble lipase, respectively. Both soluble lipase and lipase expressed on bacteriophages reacted covalently with a phosphonate suicide inhibitor. The phage does not hamper lipase binding, since both soluble and phage-bound lipase have a similar half-life of inactivation of approximately 5 min. Therefore, we conclude that the Bacillus lipase can be functionally expressed on bacteriophages as a fusion to the phage coat protein g3p. The specific interaction with the suicide inhibitor offers a fast and reproducible method for the future selection of mutant enzymes with an enantioselectivity towards new substrates.

L5 ANSWER 7 OF 10 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 2002:72261 HCAPLUS
DOCUMENT NUMBER: 136:130776
TITLE: Cloning, sequences, enantioselectivity and use of wild-type and recombinant lipases from Bacillus
INVENTOR(S): Giver, Lorraine J.; Minshull, Jeremy; Vogel, Kurt
PATENT ASSIGNEE(S): Maxygen, Inc., USA
SOURCE: PCT Int. Appl., 196 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002006457	A2	20020124	WO 2001-US22160	20010713
WO 2002006457	A3	20021017		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1307548	A2	20030507	EP 2001-957149	20010713
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2003096390	A1	20030522	US 2001-905666	20010713
US 6858422	B2	20050222		
US 2005214919	A1	20050929	US 2004-920059	20040817
PRIORITY APPLN. INFO.:			US 2000-217954P P	20000713
			US 2001-300378P P	20010621
			US 2001-905666 A3	20010713
			WO 2001-US22160 W	20010713

AB New lipases (both nucleic acids and polypeptides) from different strains of Bacillus (both known Bacillus species and un-typed Bacillus species) are provided. The nucleotide sequences and the encoded amino acid sequences of the wild-type lipases detected in Bacillus or the recombinant Bacillus lipases are disclosed. Enantioselectivity of the Bacillus liases for neryl butyrate or geranyl butyrate was detd. Compns. which include these polypeptides, proteins, nucleic acids, recombinant cells, as well as methods involving the enzymes, antibodies to the enzymes, and methods of using the enzymes are also provided.

L5 ANSWER 8 OF 10 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2002:463252 HCAPLUS
DOCUMENT NUMBER: 137:259280
TITLE: Alternate conformations observed in catalytic serine of Bacillus subtilis lipase determined at 1.3 .ANG. resolution
AUTHOR(S): Kawasaki, Kosei; Kondo, Hidemasa; Suzuki, Mamoru; Ohgiya, Satoru; Tsuda, Sakae
CORPORATE SOURCE: National Institute of Advanced Industrial Science and Technology (AIST), Research Institute of Biological Resources, Structural Biology Group, Toyohira, Sapporo, 062-8517, Japan
SOURCE: Acta Crystallographica, Section D: Biological Crystallography (2002), D58(7), 1168-1174
CODEN: ABCRE6; ISSN: 0907-4449
PUBLISHER: Blackwell Munksgaard
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Bacillus subtilis extracellular lipase (BsL) has an exceptionally low mol. wt. (19.4 kDa) for a member of the lipase family. A crystallog. study was performed on BsL in order to design and produce mutant BsL that will be more suitable for industrial uses based on anal. of the three-dimensional structure. Recently, the crystal structure of BsL has been detd. at 1.5 .ANG. resoln. [van Pouderoyen et al. (2001). J. Mol. Biol. 309, 215-226]. In the present study, a new crystal form of BsL which provides diffraction data to higher resoln. was obtained and its structure was detd. at 1.3 .ANG. using the MAD method. It was found that the active-site residue Ser77 has alternate side-chain conformations . The O.gamma. atom of the first conformer forms a hydrogen bond to the N* of His155, a member of the catalytic triad. In contrast, the second

conformer is constructed with a hydrogen bond to the side-chain atom of the adjacent His76 . These two conformers presumably correspond to the active and inactive states, resp. Similar alternate conformations in the catalytic serine residue have been obsd. in Fusarium solani cutinase detd. at 1.0 .ANG. resoln. and Penicillium purpurogenum acetylxylan esterase at 0.9 .ANG. resoln. In addn., a glycerol mol., which ws used as a cryoprotectant, is found to be located in the active site. On the basis of these results, a model for substrate binding in the reaction-intermediate state of BsL is proposed.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 9 OF 10 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2001:70314 HCAPLUS
DOCUMENT NUMBER: 134:262797
TITLE: Substitution of glycine 275 by glutamate (G275E) in lipase of Bacillus stearothermophilus affects its catalytic activity and enantio- and chain length specificity
AUTHOR(S): Kim, Myung Hee; Kim, Hyung-Kwoun; Oh, Byung-Chul; Oh, Tae-Kwang
CORPORATE SOURCE: Environmental Bioresources Lab., Korea Research Institute of Bioscience & Biotechnology, Taejon, 305-600, S. Korea
SOURCE: Journal of Microbiology and Biotechnology (2000), 10(6), 764-769
CODEN: JOMBES; ISSN: 1017-7825
PUBLISHER: Korean Society for Applied Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The lipase gene (lip) from Bacillus stearothermophilus was recombined in vitro by utilizing the DNA shuffling technique. After four rounds of shuffling, transformation, and screening based on the initial rate of clear zone formation on a tricaprylin plate, a clone (M10) was isolated, the cell ext. of which showed about 2.8-fold increased lipase activity. The DNA sequence of the mutant lipase gene (m10) showed 3 base changes, resulting in two cryptic mutations and one amino acid substitution; S113 (AGC.fwdarw.AGT), L252 (TTG.fwdarw.TTA), and G275E (GGA.fwdarw.GAA). SDS-PAGE anal. revealed that the increased enzyme activity obsd. in M10 was partly caused by high expression of the m10 lipase gene. The amt. of the expressed G275E lipase was estd. to comprise as much as 41% of the total sol. proteins of the cell. The max. velocity (Vmax) of the purified mutant enzyme for the hydrolysis of olive oil was measured to be 3,200 U/mg, which was 10% higher than that of the parental (WT) lipase (2,900 U/mg). Its optimum temp. for the hydrolysis of olive oil was 68.degree.C and it showed a typical Ca2+-dependent thermostability, properties of which were the same as those of the WT lipase. However, the mutant enzyme exhibited a high enantiospecificity towards (S)-naproxen compared with the WT lipase. In addn., it showed increased hydrolytic activity towards triolein, tricaprin, tricaprylin, and tricaproin.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 10 OF 10 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1993:669189 HCAPLUS
DOCUMENT NUMBER: 119:269189
TITLE: Secretory manufacture of an heterologous lipase in Bacillus subtilis using a chimeric gene containing a Bacillus signal sequence
INVENTOR(S): Power, Scott D.; Van Kimmenade, Johanna M. A.; Carlomagno, Louann P.
PATENT ASSIGNEE(S): Genencor International, Inc., USA
SOURCE: PCT Int. Appl., 29 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9320214	A1	19931014	WO 1993-US3018	19930330
W: CA, FI, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5429950	A	19950704	US 1992-860468	19920330
CA 2133338	A1	19931014	CA 1993-2133338	19930330
CA 2133338	C	20060613		
EP 646177	A1	19950405	EP 1993-908693	19930330
EP 646177	B1	20031210		
R: BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE				
JP 07505292	T	19950615	JP 1993-517662	19930330
JP 3432224	B2	20030804		
FI 9404524	A	19940929	FI 1994-4524	19940929

PRIORITY APPLN. INFO.: US 1992-860468 A 19920330
WO 1993-US3018 W 19930330

AB An heterologous lipase is manufd. in Bacillus as a fusion protein with a short N-terminal signal peptide derived from a Bacillus protein using an expression construct integrated into the host chromosome. Secondary mutations in other genes may be introduced to improve efficiency of secretion. The preferred lipase is cutinase of Pseudomonas mendocina and the preferred Bacillus protein is the aprE gene product. This method allows the manuf. of proteins from Gram-neg. bacteria in Bacillus. A series of chimeric genes for cutinase with aprE signal peptides and intervening peptides of different lengths were constructed and introduced into B. subtilis and tested for levels of cutinase in the medium. Cutinase was detected in the medium with the most effective constructs yielding medium cutinase with prodn. rates up to 5-fold greater than the simplest of the construct.

=> d his

(FILE 'HOME' ENTERED AT 14:59:53 ON 15 FEB 2007)

FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, SCISEARCH, EMBASE' ENTERED AT 15:00:41 ON 15 FEB 2007

L1 69 S LIPASE GENE VARIANT?
L2 31 DUP REM L1 (38 DUPLICATES REMOVED)
L3 1 S L2 AND BACILLUS
L4 18 S BACILLUS LIPASE AND (MUTANT? OR VARIANT? OR MUTATION?)
L5 10 DUP REM L4 (8 DUPLICATES REMOVED)
L6 0 S L5 AND N166Y
L7 0 S L5 AND A69V

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	40.17	40.38

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-5.46	-5.46

STN INTERNATIONAL LOGOFF AT 15:06:14 ON 15 FEB 2007

<!--StartFragment-->RESULT 1
ADR23264
ID ADR23264 standard; protein; 181 AA.
XX
AC ADR23264;
XX
DT 04-NOV-2004 (first entry)
XX
DE Bacillus subtilis lipase N166Y mutant.
XX
KW Lipase; enzyme; thermostable; protein engineering; mutant; mutein; ss.
XX
OS Bacillus subtilis.
OS Synthetic.
XX
FH Key   Location/Qualifiers
FT Misc-difference 103
FT   /note= "Encoded by GTT"
FT Misc-difference 154
FT   /note= "Encoded by GGCGTT"
FT Misc-difference 166
FT   /note= "Wild-type Asn substituted by Tyr"
XX
PN WO2004067705-A2.
XX
PD 12-AUG-2004.
XX
PF 29-JAN-2004; 2004WO-IN000022.
XX
PR 30-JAN-2003; 2003IN-DE000075.
XX
PA (COUL) COUNCIL SCI & IND RES.
XX
PI Rao NM, Acharya P;
XX
DR WPI; 2004-594198/57.
DR N-PSDB; ADR23278.
XX
PT Novel lipase gene variants that is thermostable, organic solvent
PT resistant and high pH tolerant, useful in household detergents and
PT laundry industry.
XX
PS Claim 1; SEQ ID NO 2; 51pp; English.
XX
CC The present sequence is the protein sequence of a Bacillus subtilis
CC lipase LipA mutant in which the native Asn residue at position 166 is
CC substituted by Tyr. The mutant enzyme has a molecular weight of 19443,
CC and is a claimed example of novel thermostable, organic solvent resistant
CC and high pH tolerant lipase variants of the invention. It was produced
CC using a directed evolution methodology. A claimed method of preparing an
CC expression system of novel thermostable, organic solvent resistant and
CC high pH tolerant lipase gene variants, including the present variant,
CC comprises: isolating and purifying the lipase gene from B. subtilis;
CC cloning the gene in vector pJO290; generating gene variants from the
CC lipase gene by random mutagenesis and site-directed mutagenesis using
CC forward primer JOF ADR23275 and reverse primer JOR ADR23276; cloning the
CC gene variants in vector pJO290; and ligating the cloned gene variants in
CC Escherichia coli JM109. The novel lipase variants are thermostable in the
CC temperature range 55-90 degrees C, are resistant to organic solvents such
CC as acetonitrile, isopropanol, dimethyl sulfoxide and dimethyl formamide,
CC have the ability to withstand high pH in the range 9-13, and also have
CC the ability to withstand proteases and damaging surfactants comprising
CC linear alkyl benzene sulfonates. They can be used as stain removers in

SEQ ID NO: 2.

A69V/A133D/N166Y — SEQ 3

A69T/L115P/N166Y — SEQ 4

N (Asparagine)

A133D/N166Y → SEQ 5

L115P/A133D/N166Y → SEQ 6

Table 2

45/190

CC household detergents and the laundry industry. The present lipase variant
CC retains 30.9% of initial activity after 60 minutes at 55 degrees C,
CC whereas wild-type LipA has 36.02% residual activity after 7.5 minutes. It
CC has a Km value of 1.03 mM (0.97 for wild-type LipA), a kcat value of 5.0
CC x 10 -2/min (5.2 x 10 -2/min) and a half-life at 55 degrees and pH 7.0 of
CC 25.4 minutes (2.5 minutes). Residual activity after 30 minutes in 20%
CC acetonitrile is 28.7% (22% for the wild-type).
XX
SQ Sequence 181 AA;

Query Match 100.0%; Score 946; DB 8; Length 181;
Best Local Similarity 100.0%; Pred. No. 3.7e-90;
Matches 181; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

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Qy          1 MAEHNPVVMVHGIGGASFNFAGIKSYLVSQGWSRDKLYAVDFWDKTGTNYNNGPVLSRFV 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MAEHNPVVMVHGIGGASFNFAGIKSYLVSQGWSRDKLYAVDFWDKTGTNYNNGPVLSRFV 60

Qy         61 QKVLDETGAKKVDIVAHSMGGANTLYYIKNLDGGNKVANVVTLGGANRLTTGKALPGTDP 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 QKVLDETGAKKVDIVAHSMGGANTLYYIKNLDGGNKVANVVTLGGANRLTTGKALPGTDP 120

Qy        121 NQKILYTSIYSSADMIVMNYLSRLDGARNVQIHGGHIGLLYSSQVYSLIKEGLNGGGQNT 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 NQKILYTSIYSSADMIVMNYLSRLDGARNVQIHGGHIGLLYSSQVYSLIKEGLNGGGQNT 180

Qy        181 N 181
              |
Db        181 N 181
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<!--EndFragment-->